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Directional Instability of Microtubule Transport in the Presence of Kinesin and Dynein, Two Opposite Polarity Motor Proteins

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Abstract. Kinesin and dynein are motor proteins that move in opposite directions along microtubules. In this study, we examine the consequences of having kinesin and dynein (ciliary outer arm or cytoplasmic) bound to glass surfaces interacting with the same microtubule in vitro. Although one might expect a balance of opposing forces to produce little or no net movement, we find instead that microtubules move unidirectionally for several microns (corresponding to hundreds of ATPase cycles by a motor) but continually switch between kinesin-directed and dynein-directed transport. The velocities in the plus-end (0.2–0.3 μm/s) and minus-end (3.5-4 μm/s) directions were approximately half those produced by kinesin (0.5 μm/s) and ciliary dynein (6.7 μm/s) alone, indicating that the motors not contributing to movement can interact with and impose a drag upon the microtubule. By comparing two dyneins with different duty ratios (percentage of time spent in a strongly bound state during the ATPase cycle) and varying the nucleotide conditions, we show that the microtubule attachment times of the two opposing motors as well as their relative numbers determine which motor predominates in this assay. Together, these findings are consistent with a model in which kinesin-induced movement of a microtubule induces a negative strain in attached dyneins which causes them to dissociate before entering a force-generating state (and vice versa); reversals in the direction of transport may require the temporary dissociation of the transporting motor from the microtubule. The bidirectional movements described here are also remarkably similar to the back-and-forth movements of chromosomes during mitosis and membrane vesicles in fibroblasts. These results suggest that the underlying mechanical properties of motor proteins, at least in part, may be responsible for reversals in microtubule-based transport observed in cells.

MANY forms of cytoplasmic motility, such as mitotic chromosome segregation, the transport of vesicular organelles, and the beating of cilia and flagella, are generated by interactions between microtubules and a variety of mechanochemical enzymes termed "motors" (Schroer and Sheetz, 1991; Vallee and Shpetner, 1990). Motors move unidirectionally along the asymmetric microtubule; the information for directional motion is presumably specified by the stereospecific interactions between the motor and the tubulin monomers in the microtubule lattice. Conventional kinesin is a plus-ended directed motor (Vale et al., 1985), whereas cytoplasmic dynein (Paschal and Vallee, 1987), axonemal dynein (Sale and Satir, 1977; Vale and Toyoshima, 1988) and the kinesin-like motor *ncd* (McDonald et al., 1990; Walker et al., 1990) all direct movement towards the minus end. Having two classes of polarity-specific motors enables cells to transport subcellular structures either towards or away from its center on a unipolar microtubule array nucleated from the centrosome.

Continuous movement in one direction is important if subcellular structures are to be transported efficiently over long distances. Hence, vesicles undergoing axonal transport between the cell body and nerve terminal tend to move continuously in either the plus-end (anterograde) or minus-end (retrograde) direction, and rarely reverse their polarity of movement (Allen et al., 1982). On the other hand, individual vesicles in smaller cells, such as fibroblasts, frequently switch between plus-end and minus-end directed transport (Herman and Albertini, 1984; Rebhun, 1964; Roos et al., 1987), which may favor chance encounters between vesicles and their target membranes. Chromosomes also frequently and abruptly switch their polarity of movement along microtubules during prometaphase, metaphase and even anaphase in some cell types (Bajer, 1982; Rieder et al., 1986). The oscillatory movements during prometaphase are thought to play a key role in aligning chromosomes at the metaphase plate before anaphase segregation of the sister chromatids.

Reversals in the direction of movement by chromosomes and vesicles indicate that these subcellular structures have both plus- and minus-end directed motors bound to their surfaces. Although the mechanism that allows chromosomes and vesicles to switch abruptly between plus- and minus-end motility is not understood, it is generally thought that the activities of the opposite polarity motors must be controlled in a coordinated manner, perhaps by post-translational modifications. In support of this idea, agents that affect phosphory-

lation state influence the polarity of movement of pigment granules along microtubules in melanophores (Rozdzial and Haimo, 1986) and of isolated chromosomes along microtubules in vitro (Hyman and Mitchison, 1991). Whether a coordinated switch is required for reversing the direction of movement, however, is unclear.

In this study, we used a simplified in vitro motility assay to examine the consequences of having two opposite polarity motors (kinesin and dynein) generating force on the same microtubule. Although we expected the opposing forces to produce slowly moving or stationary microtubules when balanced, we instead found that microtubules continually switch between plus- and minus-end directed transport but can undergo unidirectional excursions of several microns. By studying this phenomenon using two different types of dynein (ciliary and cytoplasmic), different ratios of kinesin to dynein, and different nucleotide conditions, we have gained insight into the properties of kinesin and dynein that allow switching in the direction of transport to occur. The mechanical properties of motor proteins that produce bidirectional transport in vitro might also be responsible, at least in part, for the abrupt and frequent reversals of chromosome and vesicle movements in living cells.

Materials and Methods

Preparation of Kinesin, Dynein and Microtubules

Squid optic lobe kinesin was prepared by microtubule affinity, as previously described (Vale et al., 1985*a*), and then further purified by sucrose density gradient sedimentation (5–20% sucrose density gradient prepared in 80 mM Pipes (pH 6.8), 1 mM $MgCl_2$, 1 mM EGTA; 200,000 g_{max}/12 h). Squid optic lobe dynein was prepared essentially by the method of Schnapp and Reese (Schnapp and Reese, 1989). Outer arm dynein was prepared from the cilia of *Tetrahymena thermophila*, as described previously (Johnson, 1986; Vale and Toyoshima, 1988). *Tetrahymena* outer arm dynein and the squid optic lobe kinesin were frozen and stored in liquid nitrogen. The squid cytoplasmic dynein was generally kept on ice and used within a week after its preparation. The protein concentrations of kinesin and cytoplasmic dynein were estimated by comparing the Coomassie blue staining intensity of their heavy chain polypeptides to BSA standards, as described by Hackney (1988). The protein concentration of *Tetrahymena* outer arm dynein was estimated by measuring the adsorbance at 280 nm and using an extinction coefficient of 0.97 cm^2/mg (Johnson, 1986). The molecular weights of the kinesin and dynein heavy chains were taken as 120 and 500 kD, respectively; the molecular weights used for the entire kinesin, cytoplasmic dynein and ciliary dynein molecules were 360, 1,200 and 1,960 kD, respectively.

Tubulin was purified by cycles of polymerization and depolymerization followed by phosphocellulose chromatography (Mitchison and Kirschner, 1984) and was modified with tetramethylrhodamine succinimidyl ester to a stoichiometry of ~1 fluorochrome per tubulin dimer (Hyman et al., 1990). Fluorescent, polarity-marked microtubules were prepared essentially as described by Hyman (1991). First, stable microtubule seeds were prepared by polymerizing rhodamine-labeled tubulin (2 mg/ml) in the presence of 0.5 mM guanylyl-(α,β)-methylene-diphosphate (GMPCPP) (obtained from A. Hyman and T. Mitchison, University of California, San Francisco) in 80 mM Pipes (pH 6.8), 1 mM $MgCl_2$, 1 mM EGTA. These seeds were then used at a concentration of 0.17 mg/ml to nucleate microtubule assembly (12 min at 37°C) in the above buffer containing 1 mM GTP, 0.3 mg/ml rhodamine-labeled tubulin, 1.7 mg/ml unlabeled tubulin, and 1.2 mg/ml N-ethyl-maleimide-modified tubulin (NEM). NEM-modified tubulin (prepared as described by Vale and Toyoshima, 1988) was included to reduce or eliminate the polymerization of tubulin from the minus-ends of the seeds. After polymerization, the polarity-marked microtubules were diluted to a concentration of 0.25–0.5 mg/ml in the appropriate motility buffer (see below) containing 10 μM taxol (obtained from the National Cancer Institute, Bethesda, Maryland).

Microtubule Translocation Assay

A microscope perfusion chamber (~6 μl) was prepared which consisted of two narrow strips of double-stick adhesive tape that formed a seal between a glass slide and an 18 × 18 mm (no. 1) coverglass. Fluids were exchanged by pipetting at one end and wicking at the other end with Whatman filter paper. In the motility assays with kinesin and cytoplasmic dynein, the surfaces of the chamber were first precoated with casein (5 mg/ml for 2 min). Casein works more reliably than cytochrome *c*, which was used in previous low density motor assays (Howard et al., 1989). After removing the unbound casein by perfusion with 40 μl of 80 mM Pipes (pH 6.8), 1 mM $MgCl_2$, 1 mM EGTA), one chamber volume of cytoplasmic dynein or kinesin were perfused into the flow cell. Ciliary dynein (90 μg/ml) was adsorbed directly onto the glass surfaces of the microscope chamber without a precoating of casein. Dual motor assays were performed by adsorbing kinesin over surfaces coated either with cytoplasmic or ciliary dynein prepared as indicated above. In all instances, motors were adsorbed to the surfaces for 3 min, and the unbound motors were washed out of the chamber by perfusion with 20 μl of buffer. The assay buffer for cytoplasmic dynein was 80 mM Pipes (pH 6.8), 1 mM $MgCl_2$, 1 mM EGTA; ciliary outer arm dynein was assayed in 50 mM K-acetate, 3 mM $MgCl_2$, 1 mM EGTA, 10 mM MES (pH 6.5), 0.1% Triton X-100 (Surfact-Amps X-100, Pierce Chemical Co., Rockford, IL). Kinesin motility was equivalent in both buffers, hence the assay buffer for dual motor experiments was dictated by the dynein species. Polarity-marked fluorescent microtubules (5–10 μg/ml) were then introduced into the chamber in the appropriate motility buffer containing 1 mM ATP, an ATP regenerating system (2 mM phosphocreatine and 100 μg/ml creatine kinase), and an oxygen depletion system (Kishino and Yanagida, 1988), which is important for preventing photo-damage to the motors during observation by fluorescence microscopy.

Adsorption of Motors to Surfaces

To determine the surface density of motors, it was necessary to determine their adsorption efficiency to the protein-coated glass. Because adsorption efficiency was best ascertained at low concentrations of motors, a bioassay described by Howard et al. (1989) was used for this determination. Essentially, a 1 μg/ml solution of either kinesin or cytoplasmic dynein was adsorbed to the surface of the chamber for 3 min. The solution within the chamber was obtained by perfusing with a pipetteman at one end and collecting the contents with a pipetteman at the other end. This solution was then adsorbed onto a second casein-coated perfusion chamber; the amount of microtubule movement produced in the second chamber was compared to that elicited by various concentrations of kinesin or cytoplasmic dynein directly adsorbed onto casein-coated surfaces. This assay revealed an almost quantitative adsorption of kinesin to cytoplasmic dynein (100%), ciliary dynein (95%) and casein (90%) -coated surfaces. Cytoplasmic dynein was adsorbed somewhat less efficiently to the casein-coated surfaces (70%). An adsorption efficiency of >90% of ciliary dynein to the glass surface was previously determined (Vale and Toyoshima, 1989). A surface density of the motors was estimated from the adsorption efficiency, the solution concentration of motor, and the surface area of the perfusion chamber. Only a subset of the absorbed motors, however, is probably competent for producing microtubule translocation, and this percentage probably varies with different types of motors. The peak fraction of the cytoplasmic dynein contains a trace contaminant of kinesin (~100-fold lower protein concentration than cytoplasmic dynein) which could be detected by immunoblot or by motility assay but not by Coomassie staining of polyacrylamide gels. This minor contaminant of kinesin absorbs with great efficiency and activity to the casein-coated surface, and as as consequence, single motor kinesin movement is also detected in the peak cytoplasmic dynein fraction. At very low densities of cytoplasmic dynein fractions, however, the vast majority (90%) of microtubule movement was minus-end directed. This problem was reduced but not entirely solved by adsorbing the kinesin in the cytoplasmic dynein fraction to a monoclonal antibody against squid kinesin (G-39 (Kosik et al., 1990), obtained from Dr. Bruce Schnapp, Harvard University, Boston, MA) attached to protein A-Sepharose beads. In dual motor kinesin-cytoplasmic dynein assays, the calculation of kinesin density incorporates the contaminating kinesin in the cytoplasmic dynein fraction as well as any added kinesin.

Video Microscopy and Analysis

Fluorescence microscopy was performed on a Zeiss IM-35 microscope

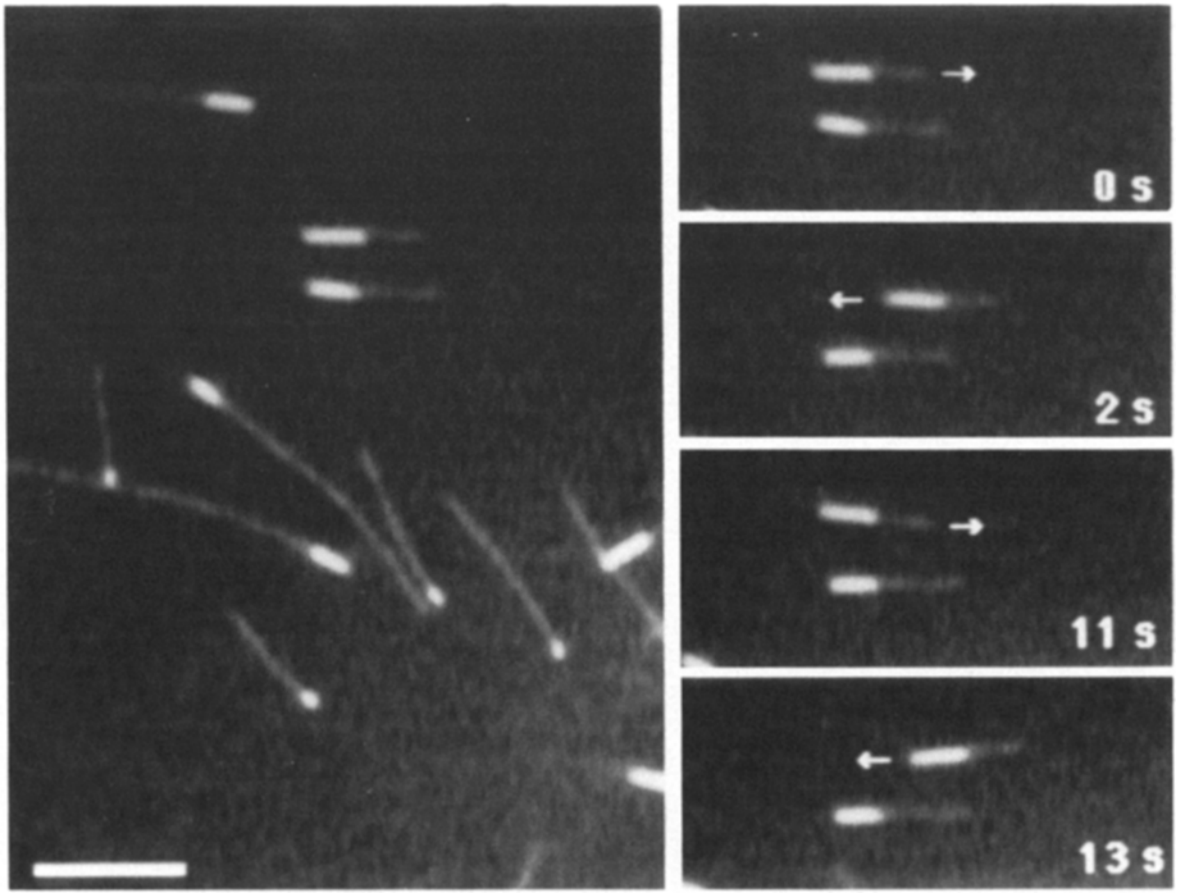


Figure 1. Microtubules undergo plus- and minus-end directed movements on surfaces coated with kinesin (10 molecules/μm^2) and ciliary dynein (900 molecules/μm^2). The left shows a field of polarity-marked microtubules viewed by fluorescence microscopy and imaged using a silicon-intensified target camera. The bright segment denotes the minus-end of the microtubule. The horizontally oriented pair of microtubules are followed over a period of 13 s in the panels on the right. The lower microtubule is immotile and thus provides a stationary reference point. (Approximately 10–15% of the microtubules on dynein-coated surfaces, with or without a sparse density of kinesin, are immotile, perhaps due to tight binding interactions with inactive dynein molecules). Between 0 and 2 s, the bright (minus) end of the microtubule is trailing in the direction of movement, indicating minus-end directed transport by dynein motors attached to the glass. From 2 to 11 s, the microtubule is undergoing kinesin-directed transport, and between 11 and 13 s, the microtubule has switched again to dynein movement; kinesin motility resumed at 13 s. Bar, 3 μm.

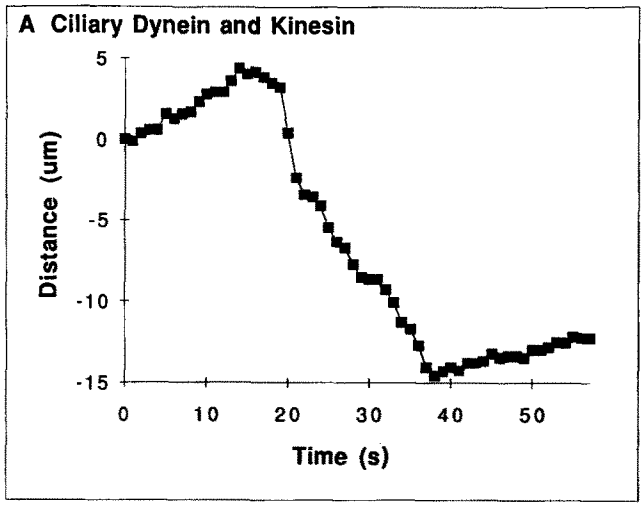
(Carl Zeiss, Inc., Thornwood, NY) using a 63×, 1.4 n.a. planapochromatic objective lens with illumination provided by a 100 W mercury light source through an epifluorescence pathway. The image was projected either via a 10× or 20× eyepiece to a Hamamatsu silicon-intensified target camera (Hamamatsu Corp., Bridgewater, NJ) and was then stored onto 1/2 inch VHS tape using a Panasonic NV-8950 tape recorder. A single field of view was recorded for <3 min, so as avoid possible photo-damage. Velocities of microtubule movement were analyzed using an interactive computer program written by Dr. Steve Block (Sheetz et al., 1986). The records of microtubule displacement were made by tracking the end of the microtubule using a custom software program developed in this laboratory.

Results

Microtubule Movement on Surfaces Coated with Kinesin and Ciliary Dynein

The in vitro motility assays used in this study involve coating glass surfaces with motors and then observing the movement of polarity-marked fluorescent microtubules across the surface (Hyman, 1991). These microtubules contain a strongly fluorescent segment at their minus-ends and a more dimly fluorescent segment at their plus-ends (Fig. 1). On kinesin or dynein coated coverslips, the bright fluorescent portion is leading or trailing in the direction of movement respectively.

After adsorption of kinesin at increasing densities onto a surface coated with ciliary dynein, we expected minus-end directed movement to slow down gradually and then come to a virtual halt at some critical kinesin concentration. Unexpectedly, however, the ciliary dynein and kinesin forces could not be balanced in such a way as to make all the microtubules stationary. Instead, microtubules would switch continually between plus-end and minus-end directed movement (Figs. 1 and 2 *a*). This behavior was particularly evident at kinesin densities between 2.5–10 molecules/μm^2 (Fig. 3; densities were estimated as described in Materials and Methods, but the proportions of active versus nonactive motors on the surface are unknown). Continuous movement in either the kinesin or dynein directions could occur for several microns before a switching event took place. The transitions between kinesin and dynein movement generally, although not always, occurred abruptly. There were also occasions when a microtubule appeared stationary or switched between dynein and kinesin motion over short distances (<1 μm) (not shown).



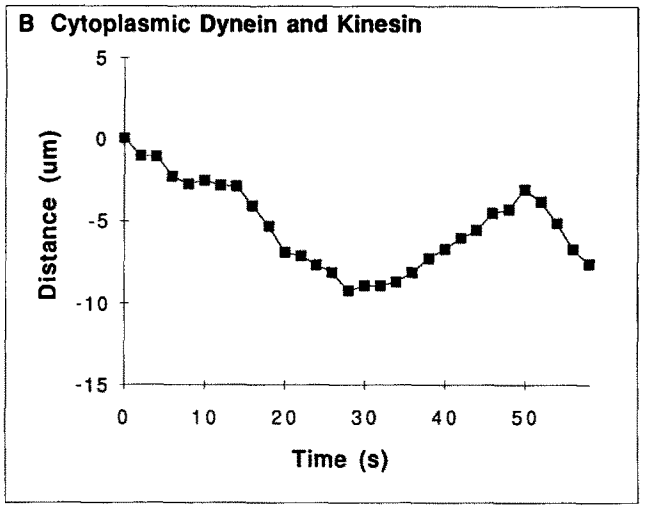


Figure 2. Movement of microtubules on surfaces coated with kinesin and either ciliary dynein (*A*) or cytoplasmic dynein (*B*). In both cases, increasing values of displacement correspond to kinesin-directed motility, whereas decreasing values correspond to dynein-directed motility. This graph illustrates that long unidirectional excursions can occur before the microtubule reverses its direction of movement. The overall behavior is similar with cytoplasmic and ciliary dynein, although the velocities of minus-end directed motility are different (2.9 μm/s for ciliary dynein and 0.5 μm/s for cytoplasmic dynein; kinesin motion occurred at $\sim$0.3 μm/s in both cases). Smaller back-and-forth movements (<1 μm) can also be seen on visual inspection, but are difficult to measure accurately with our analysis system. The lengths of microtubules were 14.4 μm (*A*) and 6.5 μm (*B*). The surface densities of kinesin in *A* and *B* were 10 and 3 molecules/μm^2, respectively; the densities of ciliary dynein and cytoplasmic dynein were 900 and 12 molecules/μm^2, respectively.

The transitions between kinesin- and dynein-directed motility did not occur repeatedly at the same locations on the surface (which could happen if the microtubule ran off a track of motors). We also examined whether they occurred at random or with any regular timing, by determining the number of times that microtubules switched direction during 10 s time intervals. The distribution of switching events in one assay (no events, 73; one event, 35; two events, 10; three events, 4; four events, 2; five events, 1) was not dissimilar to that expected by Poisson statistics (no events, 66; one event, 42; two events, 13; three events, 3; four events, 1; five events, zero). These results suggest that switching in the direction of transport occurs by a stochastic process.

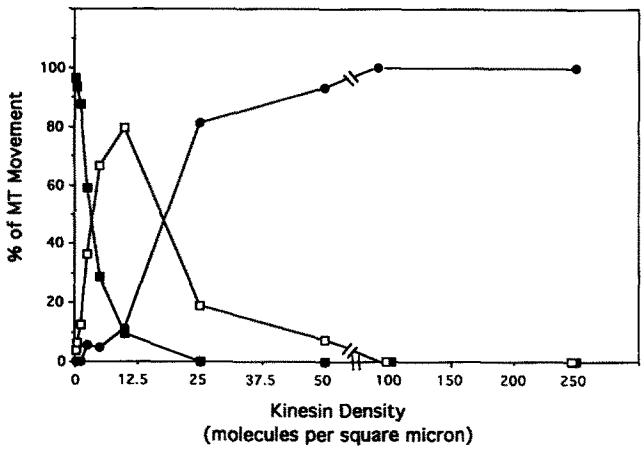


Figure 3. The polarity of microtubule movement is dependent upon the kinesin density. Kinesin was adsorbed onto a surface of ciliary dynein (900 molecules/μm^2) at the indicated densities. Microtubules that could be observed either for a period of 1 min or for a distance of travel of >30 μm were scored for their direction of movement (kinesin direction, ●; dynein direction, ■; bidirectional, □). Bidirectional microtubules are those which switched direction at least once during the observation period. Between 100–200 microtubules were analyzed at each density. % of MT movement refers to the percentage of moving microtubules that exhibited the indicated direction(s) of transport relative to all those scored. At very low kinesin densities (2.5 molecules/μm^2), the length distribution of the microtubules that moved bidirectionally (7.9 ± 3.5 μm) was similar to the overall microtubule population (7.6 ± 5.6 μm). At higher kinesin densities (50 molecules/μm^2), microtubules that exhibited bidirectional motion (2.5 ± 1.0 μm) were shorter than the overall population (5.5 ± 3.6 μm).

Bidirectional movement could be produced if microtubules encountered patches of dynein or kinesin motors and moved unidirectionally for long distances when interacting with a single motor species. However, velocity measurements indicated that bidirectionally moving microtubules were in contact with both motors. Under conditions where bidirectional transport was commonly observed (0.25–10 kinesin molecules/μm^2), the velocity in the plus-end direction (0.2–0.3 μm/s) was approximately half that produced by kinesin alone (0.5 μm/s) (Fig. 4). The velocities of microtubules that switched from minus- to plus-end movement were similar to those that moved continuously in the plus-end directed direction during the observation period. Similarly, the minus-end velocities (3.5–4 μm/s) were approximately one-half that produced by dynein in the absence of kinesin (6.7 μm/s). The decreased kinesin and dynein velocities indicate that the opposite polarity motors impose a drag on the moving microtubule. Thus, both kinesin and dynein seem to interact with the microtubule, and the active force-generating species appears to switch back-and-forth by an apparently stochastic process.

The decreased velocity of movement imposed by opposite polarity motors could be caused by motors being dragged along the surface; however, a more likely source of this drag is a "protein friction" (Tawada and Sekimoto, 1991) caused by stationary motors slipping from binding site to binding site along the microtubule. To explore further whether a weak or a strong binding state between the motor and the microtubule is responsible for this drag, ciliary dynein was

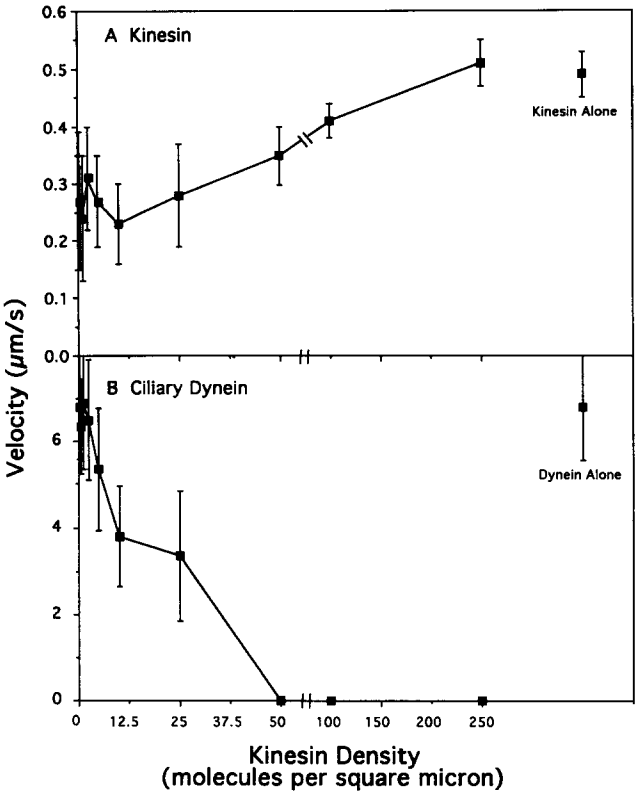


Figure 4. The velocities of kinesin (*A*) and ciliary dynein (*B*) motility at different kinesin densities on a ciliary dynein coated surface. The squares indicate the mean and the error bars represent the standard deviations of velocity measurements from between 10 and 40 microtubules. These data were derived from the same experiment shown in Fig. 3. At kinesin densities where bidirectional motion predominated, the plus- and minus-end directed velocities were approximately one-half of those elicited by kinesin or ciliary dynein alone.

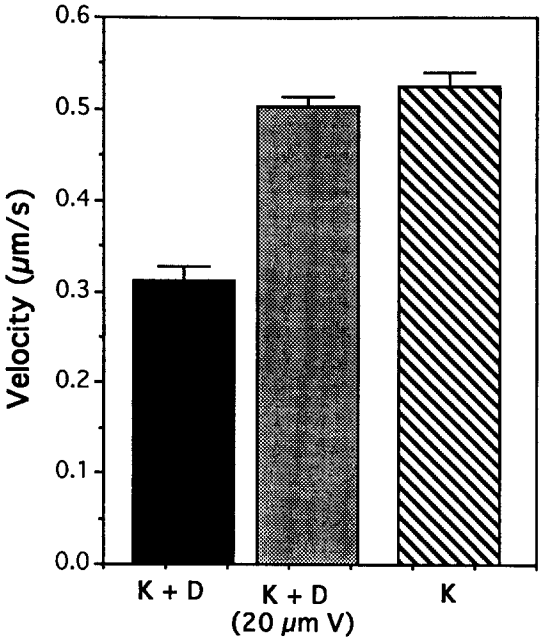


Figure 5. Vanadate eliminates the ciliary dynein-mediated decrease in kinesin velocity. The mean velocities and standard errors are shown for thirty microtubules moving in the plus-end direction on surfaces coated with kinesin (10 molecules/μm^2) and ciliary dynein (900 molecules/μm^2) ($K + D$) in the absence or presence of 20 μM vanadate. The velocities under these conditions are significantly different from one another ($p < 0.001$). The velocities in the presence of vanadate are not significantly different from those elicited by kinesin alone (K) ($p > 0.2$). These results indicate that a weak binding (ADP-vanadate) state of dynein does not impede kinesin-driven motility.

trapped by the phosphate analogue vanadate in a weak binding state (ADP-vanadate), which causes microtubules to bind to dynein-coated coverslips and undergo one-dimensional diffusion but not directed translocation (Vale et al., 1989). Because of the different sensitivities of dynein and kinesin to vanadate (kinesin movement is unaffected by 20 μM vanadate), the effect of the ADP-vanadate dynein weak binding state on kinesin movement could be assessed (Fig. 5). At a kinesin density of 10 molecules/μm^2 on the ciliary dynein coated surface, microtubules moved in the kinesin direction at a rate of 0.31 μm/s (Fig. 5), which was significantly different from that produced by kinesin alone ($p < 0.001$). When 20 μM vanadate was added, minus-end movement was suppressed, and the velocity of the plus-end movement increased to 0.50 μm/s (not significantly different than that of kinesin alone; $p > 0.2$). Thus, a weak microtubule binding state of dynein does not produce sufficient drag to impede the velocity of kinesin-induced transport.

To gain further insight into how bidirectional movement might be generated, it is important to ascertain how many kinesin and dynein motors are interacting with the microtubule. Previous studies showed that single kinesin molecules can transport microtubules for several micrometers (Howard et al., 1989). The ability of a single kinesin to transport a microtubule, amidst considerable Brownian motion acting to separate the motor from the filament, indicates that this motor spends the majority of time tightly associated with microtubules. The percentage of the ATPase cycle spent by a motor in a strongly bound state is referred to as the "duty ratio"; in the case of kinesin, this value is very close to unity (Block et al., 1990; Howard et al., 1989). In the dual motor assay, several results also suggest that an interaction of microtubules with a single kinesin molecule is sufficient to elicit the plus-end component of bidirectional transport. First, a small percentage (3.7%) of microtubules moved bidirectionally (defined as at least one switch between plus- and minus-end directed motion occurring during a 1 min observation period) at the lowest kinesin densities tested (0.25 molecules/μm^2) (Fig. 3). Movement at these kinesin densities in the absence of dynein is rare and virtually always produced by single motors. Second, as the kinesin surface density was raised from 0.25 to 10 molecules/μm^2, the percentage of microtubules exhibiting bidirectional movement increased approximately in a linear manner. As encounters between microtubules and kinesins should increase in direct proportion to the motor surface density (Howard et al., 1989), this result suggests that bidirectional movement requires an interaction with only one kinesin.

On the other hand, a sharp transition from bidirectional movement to plus-end only movement occurred within a very narrow range of kinesin density (Fig. 3), suggestive of a cooperative interaction amongst kinesins. At kinesin densities where plus-end movement begins to predominate (25–50 molecules/μm^2), microtubules often moved dis-

tances greater than their own length, a phenomenon indicative of an interaction with two or more kinesin motors. At these densities, only the shorter microtubules in the population (<6 μm) exhibited episodes of bidirectional motion, presumably because they encountered fewer kinesins on the surface. Thus, interactions with relatively few kinesins appear to suppress ciliary dynein-directed motility.

The number of ciliary dynein molecules (900 molecules/μm²) interacting with microtubules in this assay is more difficult to assess, because a quantitative dilution assay for ciliary dynein is not available. However, as 1-μm microtubules frequently moved distances >1 μm on the surface, it seems reasonable to conclude that there must be at least one ciliary dynein molecule situated per μm length of microtubule. Thus, a 10 μm microtubule that undergoes bidirectional motion may interact with one kinesin molecule and as many as ten ciliary dyneins.

How can a single kinesin molecule overcome so many ciliary dyneins? One possibility is that kinesin generates much more force and hence is a more effective motor. However, the force generated by a single axonemal dynein has been estimated to be ~1 pN (Kamimura and Takahashi, 1981), a value which is similar to those measured for myosin (Kishino and Yanagida, 1988) and kinesin (Kuo and Sheetz, 1990). Alternatively, a difference in the duty cycle ratios between kinesin and ciliary dynein may account for the disproportionate numbers of these opposite polarity motors. In this regard, several features of the movement produced by ciliary dynein seem to suggest that it, unlike kinesin, is a low duty ratio motor. First, short microtubules (<4 μm) generally exhibited episodic translocation on dynein-coated surfaces, which differs from the smooth movement induced by kinesin. In between episodes of directed motion, microtubules would undergo one-dimensional diffusion (Vale et al., 1989), indicating that they were still maintaining contact with dynein molecules. Microtubule movement also is not observed when ciliary dynein is adsorbed at low densities on casein-coated surfaces, possibly because a single ciliary dynein molecule by itself is insufficient to maintain attachment to a microtubule for an extended period of time. Together, these observations indicate the small numbers of dyneins do not exert force continuously upon short microtubules.

To test the idea that a difference in duty ratio influences the direction of movement in the presence of opposite polarity motors, we examined whether increasing the time spent by kinesin in a weakly-bound state would increase the likelihood of microtubules undergoing dynein-directed motion. This was accomplished by adding ADP as a competitive inhibitor to ATP, which in other studies was shown to prolong a detached state in the kinesin ATPase cycle (Romberg and Vale, 1992). At a kinesin density of 100 molecules/μm² on a ciliary dynein coated surface, 97% of the microtubules moved in the plus-end direction in the presence of 0.5 mM ATP (0.35 ± 0.06 μm/s). However, in the presence of 0.5 mM ATP and 2.5 mM ADP, 100% of the microtubules now moved in the minus-end direction. The speed of movement was 1.09 ± 0.24 μm/s, which is similar to that produced by dynein alone at these ATP and ADP concentrations (1.07 ± 0.16). When kinesin was adsorbed alone onto a casein-coated surface and tested at the same density and same ATP/ADP concentrations, longer microtubules (>3 μm) moved (0.11 ± 0.01 μm/s), but were weakly bound and frequently dissociated from the surface. This dramatic effect of ADP on the direction of microtubule movement supports the notion that the attachment times of kinesin and ciliary dynein to the microtubule, as well as their relative numbers, influences the direction of movement in this assay.

Microtubule Movement on Surfaces Coated with Kinesin and Cytoplasmic Dynein

Because ciliary dynein and kinesin are unlikely to be bound to the same substrate within a cell, we also examined microtubule movement on surfaces coated with kinesin and a cytoplasmic form of dynein involved in vesicle and chromosome transport. Cytoplasmic dynein differs from ciliary dynein in its motility, ATPase properties and polypeptide composition (Vallee and Shpetner, 1990). We find that cytoplasmic dynein, in contrast to ciliary dynein, elicits minus-end-directed motion when diluted to low densities (4 molecules/μm²) on a casein-coated surface. At these cytoplasmic dynein densities, microtubules often move while pivoting about a nodal point of attachment to the surface and generally move for distances less than their length before dissociating into solution. Because these features are characteristic of motility produced by single kinesin molecules (Howard et al., 1989), we suspect that cytoplasmic dynein, like kinesin, is a high duty ratio motor, although additional analyses must be performed to confirm this hypothesis.

When kinesin was present together with cytoplasmic dynein at low density on the casein-coated surface, >85% of the microtubules switched between minus-end directed and plus-end directed transport during a 1 min observation period. Similar to the findings with kinesin and ciliary dynein, excursions of several microns in the plus- or minus-end directions could occur before transport abruptly switched to the opposite direction (Fig. 2 *B*). Microtubules would also occasionally pause or move back-and-forth over relatively short distances.

Bidirectional movement occurred over a range of kinesin and cytoplasmic dynein densities on the surface. At the lowest densities (kinesin, 0.5–1 molecules/μm²; cytoplasmic dynein, 6 molecules/μm²), such movement appeared to be due to the actions of single kinesin and cytoplasmic dynein molecules, because microtubules generally did not move distances greater than their own length in either direction. At 10–20-fold higher densities (10–20 kinesins/μm²; 60–120 cytoplasmic dyneins/μm²), bidirectional movement probably involves several opposite polarity motors interacting with the same microtubule. The instability in the direction of microtubule transport becomes suppressed when very large numbers of opposite polarity motors participate in the movement. At very high surface densities of motors (e.g., 2,000 kinesins/μm²; 1,200 cytoplasmic dyneins/μm²), microtubules moved unidirectionally at relatively slow velocities (<0.1 μm/s), the polarity depending upon the precise amounts of kinesin and cytoplasmic dynein (not shown).

Discussion

Instability in the Direction of Microtubule Transport

In the presence of plus- and minus-end directed motors, one might expect microtubules to stall or move unidirectionally, their speeds and polarity of movement being dictated by the

relative numbers and strengths of the opposing motors. Although such a situation appears to occur when large numbers of opposite polarity motors interact with a microtubule, we show here that the direction of microtubule transport becomes unstable at relatively low motor number. Microtubules can move in one direction for several microns (corresponding to hundreds of ATPase cycles by a motor) before switching to the opposite direction of transport. Persistent unidirectional transport for several microns argues that the mechanical actions of kinesin upon the microtubule inhibit dyneins from producing force and vice versa. Reversals in the direction of transport indicate that the active force-generating species can switch between kinesin and dynein by an apparently stochastic process.

What mechanism gives rise to the instability in the direction of microtubule transport? Bidirectional transport could potentially arise if one class of motor produced a global conformation change in the microtubule, which in turn inhibited force production by motors of the opposite polarity. Alternatively, this phenomenon can be explained by well-established crossbridge models (Huxley, 1957,1973) in which the attachment/detachment reactions between the motor and the filament are modulated by the strain in a springlike element within the motor. In our case, we propose that unidirectional transport of the microtubule would stretch the attached opposite polarity motors and the resulting negative strain could cause them to dissociate before they can enter into a force-generating state. Such events could happen repeatedly until the opposite polarity motors eventually engage and produce force. This model is consistent with our finding that opposite polarity motors not contributing to movement elicit a drag upon the microtubule that diminishes its velocity of transport.

To switch the direction of movement, the transporting motor presumably must dissociate for a sufficient length of time to enable the opposite polarity motor to enter a force-producing state. This may explain why the duty ratios (percentage of time spent strongly bound to the microtubule during the ATPase cycle) of the two opposing motors plays an important role in determining which motor predominates in these in vitro assays. Two or more kinesins, for example, can overcome >10 ciliary dyneins probably because kinesin spends a much greater percentage of its ATPase cycle strongly bound to a microtubule than does ciliary dynein. Thus, the product of motor number and the percentage of time spent in a strongly attached state may determine the "balance" point between two opposing microtubule motors.

Biological Implications

Many objects undergo bidirectional movements along microtubules within cytoplasm, including membrane vesicles (Herman and Albertini, 1984; Rebhun, 1964; Roos et al., 1987; Pryer et al., 1986; Weiss et al., 1986), chromosomes (Bajer, 1982; Rieder et al., 1986), and latex beads added to squid axoplasm (Vale et al., 1985*b*). The unpredictable and abrupt manner in which they change direction and the distances of unidirectional excursions (0.1–20 μm) are reminiscent of the stochastic transitions between kinesin- and dynein-directed transport observed in our in vitro assays. The bias towards poleward transport in the saltatory motion in marine eggs (Rebhun, 1964; Pryer et al., 1986) may reflect greater numbers of active minus-end directed motors compared with plus-end directed motors acting on these intracellular particles.

The unpredictable movements of chromosomes towards and away from the spindle poles have received a large degree of attention, as they are thought to be essential for the normal congression of chromosomes to the metaphase plate (Bajer, 1982; Mitchison, 1989; Rieder et al., 1986; Salmon, 1989). Such oscillatory movements could be produced by a "smart kinetochore" capable of regulating its direction of movement (Mitchison, 1989). Evidence for plus- and minus-end directed kinetochore motor proteins comes from in vitro motility assays with isolated chromosomes (Hyman et al., 1991) and centromere-binding proteins (Hyman et al., 1992) as well as immunolocation studies which show cytoplasmic dynein (Pfarr et al., 1990; Steuer et al., 1990) and a kinesin-like protein (Yen et al., 1992) localized to the kinetochore region. To produce oscillatory motion, the force-generating activities of the opposing kinetochore motors have been proposed to be controlled by a coordinated switch mechanism involving phosphorylation (Hyman and Mitchison, 1991). To achieve congression, the regulatory proteins that control kinetochore motor activity must be sensitive to their position within the spindle.

Our results suggest the possibility that oscillatory motions of chromosomes might also arise as a consequence of having two active opposite polarity motors bound at the kinetochore. Coordinated switches for the motors, although they may be required at anaphase, need not be evoked to explain the abrupt reversals in chromosome movement. Additional factors, however, must be responsible for achieving a dynamic equilibrium of chromosome position at the metaphase plate. Although the transitions between plus- and minus-end transport are stochastic in our in vitro system, nonkinetochore forces in the spindle could influence the probability of chromosomes reversing their direction of movement in vivo. For example, the increasing viscosity or forces associated with growing astral microtubules (Rieder et al., 1986) could increase the probability of a poleward moving chromosome switching to plus-end directed movement. Similarly, length-dependent traction forces (Hays et al., 1982), which grow larger as the chromosome moves away from the pole, may increase the probability of a switch from plus- to minus-end directed transport. "Communication" in the direction of movement between the two sister kinetochores (Skibbens, R. V., and E. D. Salmon, unpublished observations) may also be explained by a force-sensitive switch mechanism, because a reversal by one kinetochore would impart a force to its sister, which could trigger it to switch its direction as well. Thus, position-dependent forces, in conjunction with a random bidirectional transport process that allows chromosomes to sample their environment within the spindle, could allow congression of the oscillating chromosomes to the center of the spindle apparatus.

The above model predicts that viscous or actively opposing forces should increase the probability of switching the direction of movement. This prediction could be tested in our in vitro assay system by using optical trapping techniques (Block et al., 1990) or a flexible glass needle (Kishino and Yanagida, 1988) to apply an opposing force to the moving microtubule. However, for understanding chromosome movement, one must also take into account the dynamic instability of microtubule growth and shrinkage (Mitchison

and Kirschner, 1984), which on its own could potentially generate chromosome "oscillations". The challenge for the future will be to decipher how dynamic instability of microtubule polymerization and switching of microtubule-based motor activity might together influence the movement and activities of kinetochores in living cells.

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